

We claim:

1. A process for generating at least one partially double-stranded polynucleotide containing at least one single-stranded region at a terminal end prepared by:
 - a) providing at least one primer, P1, containing at least one labile nucleotide;
 - b) combining at least one target nucleic acid sequence with P1 to generate a double-stranded polynucleotide containing at least one labile nucleotide;
 - c) exposing the double-stranded polynucleotide to conditions that promote single-strand cleavage of the polynucleotide at the site of the at least one labile nucleotide of primer P1; and
 - d) exposing the cleaved polynucleotide to conditions that promote the dissociation of the cleaved portions of primer P1 from a terminal end.
2. The process according to claim 1, wherein the labile nucleotide is dUTP, and wherein the single-stranded cleavage of the polynucleotide at the site of the labile nucleotide occurs by treatment with uracil N-glycosylase.
3. The process according to claim 1, wherein the target nucleic acid sequence is chosen from mRNA, cDNA, genomic DNA, recombinant DNA, plasmid DNA, and amplified DNA.
4. The process according to claim 1, wherein part (b) involves the amplification of a single target nucleic acid sequence.

5. The process according to claim 1, wherein part (b) involves the amplification of 2-1000 target nucleic acid sequences.

6. The process according to claim 1, wherein at least one labile nucleotide is chosen from 5-hydroxy-2'deoxyctidine triphosphate, 5-hydroxy-2'deoxyuridine triphosphate, RNA, a photolabile base, a thermolabile base, a pH sensitive base, a chemically labile base, and an exonuclease sensitive base provided that when exonuclease is used the primer also contains at least one phosphothioate base.

7. The process according to claim 1, wherein the partially double-stranded polynucleotide contains at least one detectable label.

8. The process according to claim 7, wherein the detectable label is chosen from a radioisotope, a chromophore, a fluorophore, an enzyme, an antigen, a reactive group, and a double-stranded DNA selective reagent.

9. The process according to claim 1, wherein P1 comprises 5 to 50 nucleotides.

10. The process according to claim 9, wherein P1 comprises 10 to 30 nucleotides.

11. The process according to claim 1, wherein a second primer, P2, is used in the generation of the double-stranded polynucleotide.
12. The process according to claim 11, wherein P2 contains a detectable label.
13. The process according to claim 11, wherein P2 comprises 5 to 50 nucleotides.
14. A partially double-stranded target nucleic acid containing at least one single-stranded region at the terminal end prepared according to claim 1.
15. A method for detecting the presence or determining the amount of a target nucleic acid sequence comprising:
 - (a) preparing at least one partially double-stranded polynucleotide containing a target nucleic acid sequence and at least one single-stranded region at a terminal end according to the process of claim 1,
 - (b) hybridizing the partially double-stranded polynucleotide to one or more sets of nucleic acid probes, and
 - (c) detecting the presence or determining the amount of the hybridized partially double-stranded polynucleotide.
16. The method according to claim 15, wherein the partially double-stranded polynucleotide contains at least one detectable label.

17. The method according to claim 16, wherein the detectable label is chosen from a radioisotope, a chromophore, a fluorophore, an enzyme, an antigen, a reactive group and a double-stranded DNA selective reagent.

18. The method according to claim 15, wherein one or more sets of nucleic acid probes is attached to a solid support.

19. The method according to claim 18, wherein the solid support is chosen from capillary tubes, beads, fibers, slides, sheets, pins, microtiter plates, silicon, porous silicon, porous metal oxide, plastic, polycarbonate, polystyrene, cellulose, nitrocellulose, nylon, PVDF, glass, TEFLON[®], polystyrene divinyl benzene, aluminum, carbon, steel, iron, copper, nickel, silver, and gold.

20. The method according to claim 15, wherein one or more sets of nucleic acid probes comprise a DNA microarray.

21. A method of detecting or determining the presence or amount of at least one target nucleic acid sequence in a first sample of biological material relative to the same target nucleic acid sequence(s) in a second sample of biological material comprising:

(a) preparing at least one partially double-stranded polynucleotide containing a target nucleic acid sequence and at least one single-stranded region at a terminal end for each

target nucleic acid sequence from both the first sample of biological material and the second sample of biological material, according to the process of claim 1;

(b) hybridizing the partially double-stranded polynucleotide(s) from the first sample of biological material to one or more sets of nucleic acid probes and hybridizing the partially double-stranded polynucleotide(s) from the second sample of biological material to one or more sets of nucleic acid probes; and

(c) detecting or determining the presence or amount of partially double-stranded polynucleotide(s) from the first sample of biological material relative to the partially double-stranded polynucleotide(s) from the second sample of biological material.

22. The method according to claim 21, wherein the first sample of biological material comprises one or more cells, a cell lysate, or subcellular fraction, and the second sample of biological material also comprises one or more cells, a cell lysate, or subcellular fraction, and wherein the first and second sample differ in cell type, tissue type, physiological state, disease state, radiological, or biological treatment, or developmental stage.

23. The method according to claim 22, wherein the first sample of biological material is chosen from a cancerous cell population, and the second sample of biological material is chosen from a reference cell population of the same cell type as the cancerous cell population.

24. The process according to claim 21, wherein all of the partially double-stranded polynucleotide(s) contain at least one detectable label.

25. The method according to claim 24, wherein the detectable label is selected from a radioisotope, a chromophore, a fluorophore, an enzyme, an antigen, a reactive group, and a double-stranded DNA selective reagent.

26. The method according to claim 21, wherein one or more sets of nucleic acid probes is attached to a solid support.

27. The process according to claim 26, wherein the solid support is chosen from capillary tubes, beads, fibers, slides, sheets, pins, microtiter plates, silicon, porous silicon, porous metal oxide, plastic, polycarbonate, polystyrene, cellulose, nitrocellulose, nylon, PVDF, glass, TEFLON[®], polystyrene divinyl benzene, aluminum, carbon, steel, iron, copper, nickel, silver, and gold.

28. The method to claim 21, wherein one or more sets of nucleic acid probes comprise a DNA microarray.

29. The method according to claim 21, wherein a second primer, P2, is used in the generation of at least one partially double-stranded polynucleotide.

30. The method according to claim 29, wherein P2 contains a detectable label.

31. The method according to claim 30, wherein the P2 used for amplification of the target nucleic acid from the first sample of biological material may contain the same label or a different label than the P2 used for amplification of the target nucleic acid from the second sample of biological material.

32. The method according to claim 31, wherein the label(s) is detected by FRET.

33. A partially double-stranded polynucleotide comprising three regions, wherein:

- a) the first region comprises a single-stranded region of at least 8 nucleotides, wherein the single-stranded region was generated by:
 - (i) providing a first primer, P1, containing at least one labile nucleotide;
 - (ii) providing a second primer, P2, comprising a sequence that is specific for a target nucleic acid sequence and optionally contains a detectable label and/or a labile nucleotide;
 - (iii) amplifying the target nucleic acid sequence with P1 and P2 to generate a double-stranded amplicon containing at least one labile nucleotide;
 - (iv) exposing the double-stranded amplicon to conditions that promote single-strand cleavage of the amplicon at the site of the at least one labile nucleotide; and
 - (v) exposing the cleaved amplicon to conditions which promote the dissociation of the cleaved portions from the first region;
- b) the second region comprises the sequence of the double-stranded amplicon between the first region and a third region; and

c) the third region comprises the sequence of the double-stranded amplicon comprising P2 and the complementary sequence to P2.

34. The amplicon of claim 33, wherein the single-stranded region is at least 10 nucleotides in length.

35. The amplicon of claim 33, wherein the single-stranded region is at least 20 nucleotides in length.

36. The amplicon of claim 33 containing a detectable label.

37. The amplicon of claim 33, wherein the detectable label is selected from a radioisotope, a chromophore, a fluorophore, an enzyme, an antigen, a reactive group, and a double-stranded DNA selective reagent.

38. The amplicon of claim 33 wherein the labile nucleotide is dUTP, and wherein the single-stranded cleavage of the amplicon at the site of the labile nucleotide occurs by treatment with uracil N-glycosylase.

39. A method for generating a partially double-stranded polynucleotide containing at least one single-stranded index sequence at a terminal end comprising:

- a) preparing a first primer, Pa, comprising two regions: (i) a first region comprising a first index sequence containing at least one labile nucleotide wherein, the first region is not complementary to a target nucleic acid sequence and (ii) a second region comprising a sequence that is specific for the target nucleic acid sequence;
- b) preparing a second primer, Pb, comprising a sequence that is specific for the target nucleic acid sequence and optionally contains a detectable label and/or a second index sequence, wherein said first and second index sequences may be the same or different;
- c) amplifying the target nucleic acid sequence with Pa and Pb to generate a double-stranded amplicon from both the first sample and the second sample;
- d) exposing the amplicons to conditions that promote single-stranded cleavage of the amplicons at the site of the labile nucleotide(s);
- e) exposing the cleaved amplicons to conditions that promote the dissociation of the cleaved portions of the index region of the primer containing the labile nucleotide to generate a single-stranded region at the terminal end.

40. The method according to claim 39, wherein the double-stranded amplicon contains a detectable label.

41. The method according to claim 40, wherein the detectable label is selected from a radioisotope, a chromophore, a fluorophore, an enzyme, an antigen, a reactive group, and a double-stranded DNA selective reagent.

42. The method according to claim 39, wherein the labile nucleotide is dUTP, and wherein the single-stranded cleavage of the amplicon at the site of the labile nucleotide occurs by treatment with uracil N-glycosylase.

43. The method according to claim 39, wherein the target nucleic acid is chosen from mRNA, cDNA, genomic DNA, recombinant DNA, plasmid DNA, and amplified DNA.

44. The method according to claim 39, wherein part (c) involves the amplification of a single target nucleic acid sequence.

45. The method according to claim 39, wherein part (c) involves the amplification of 2-1000 target nucleic acid sequences.

46. A partially double-stranded amplicon containing at least one single-stranded index region at a terminal end prepared according to the method of claim 39.

47. A method for detecting or determining the presence or amount of a target nucleic acid sequence in a first sample of biological material relative to the same target nucleic acid sequence in a second sample of biological material comprising:

a) preparing a first primer, Pa, comprising two regions: (i) a first region comprising a first index sequence containing at least one labile nucleotide and wherein, the first region is not complementary to the target nucleic acid sequence and (ii) a second region comprising a sequence that is specific for the target nucleic acid sequence;

- b) preparing a second primer, Pb, comprising a sequence that is specific for the target nucleic acid sequence and optionally contains a detectable label and/or a second index sequence, wherein said first and second index sequences may be the same or different;
- c) amplifying the target nucleic acid sequence with Pa and Pb to generate a double-stranded amplicon from both the first sample and the second sample;
- d) exposing the amplicons to conditions that promote single-stranded cleavage of the amplicons at the site of the labile nucleotide(s);
- e) exposing the cleaved amplicons to conditions that promote the dissociation of the cleaved portions of the index region of the primer containing the labile nucleotide to generate a single-stranded region at the terminal end;
- f) hybridizing the single-stranded index region(s) of the partially double-stranded amplicon from the first sample of biological material to complementary single-stranded index nucleic acid sequence(s) bound to a first solid support, and hybridizing the single-stranded index region(s) of the partially double-stranded amplicon from the second sample of biological material to complementary single-stranded index nucleic acid sequence(s) bound to a second solid support, wherein said first and second solid support may be the same or different; and
- g) detecting or determining the presence or amount of the amplicon from the first sample of biological material relative to the amplicon from the second sample of biological material.

48. The method according to claim 47, wherein the solid support is chosen from capillary tubes, beads, fibers, slides, sheets, pins, microtiter plates, silicon, porous silicon, porous metal oxide, plastic, polycarbonate, polystyrene, cellulose, nitrocellulose, nylon, PVDF,

glass, TEFLON[®], polystyrene divinyl benzene, aluminum, carbon, steel, iron, copper, nickel, silver, and gold.

49. The method according to claim 47, wherein part (c) involves the amplification of 2 to 1000 target nucleic acid sequences.

50. The method according to claim 47, wherein the solid support comprises 2 to 1000 single-stranded index regions.